

WHAT IS CLAIMED IS:

1. A method for generating a *Drosophila* clipped *FRT* (cFRT) chromosome insensitive to a *P* transposase but remaining functional to a yeast site-specific flippase recombinase (FLP), comprising steps of:
  - (a) exposing a *FRT* chromosome to said *P* transposase for occurring a local and imprecise transposition, wherein said *FRT* chromosome contains a *P[FRT]* insertion with a selection marker gene;
  - (b) screening said *P[FRT]* insertion insensitive to said *P* transposase to obtain screened products;
  - (c) selecting candidate products from said screened products by further examinations; and
  - (d) exposing said candidate products by said *P* transposase and selecting a desired product by said further examinations to obtain said *Drosophila* clipped *FRT* (cFRT) chromosome is insensitive to said *P* transposase but remaining functional to yeast site-specific flippase recombinase.
2. The method according to claim 1, wherein said method further comprises a step (e) of examining the actual molecular nature of said clipped insertion by PCR (polymerase chain reaction).
3. The method according to claim 1, wherein said step (c) further comprises steps of:
  - (c1) examining said screened products for both recombination capability and homozygous viability; and
  - (c2) examining recombination accessibility of *FRT* sequences contained in a clipped *P[FRT]* insertion by the presence of said FLP to obtain said candidate products.

4. The method according to claim 3, wherein said recombination capability represents the functional activity of said clipped *P[FRT]* insertion and its homologous location relative to that of said original *P[FRT]* insertion.
5. The method according to claim 3, wherein said homozygous viability represents a genetic background after said chromosome's exposure to said *P* transposase.
6. The method according to claim 1, wherein said step (d) of exposing said candidate products by said *P* transposase and selecting said desired product by said further examinations is repeated at least twice.
7. The method according to claim 1, wherein said *Drosophila* cFRT chromosome is an isogenized homozygous viable *Drosophila* second chromosome.
8. The method according to claim 1, wherein said cFRT is formed due to a target sequence, recognized by said *P* transposase and responsible for a *P* transposase transposition, which is damaged and alternated into a type of incomplete target sequence, through one of a group consisting of:
  - (1) missing of a P5' DNA sequence region;
  - (2) missing of a P3' DNA sequence region; and
  - (3) missing of DNA sequences other than those defined in item (1) and in item (2).
9. The method according to claim 1, wherein said *Drosophila* cFRT chromosome remains the functional activity of said cFRT insertion for a site-specific recombination in the presence of said FLP.
10. The method according to claim 1, wherein an effectiveness of said *Drosophila* cFRT chromosome is monitored by a FLP-FRT system and derived modification systems thereof.

11. The method according to claim 1, wherein an effectiveness of said cFRT chromosome is monitored by molecular biology methods for the description of said cFRT DNA sequences configuration.
12. The method according to claim 1, wherein said *Drosophila* cFRT chromosome remains to behave normally as a wild type chromosome feasible for various genetic manipulations.
13. The method according to claim 1, wherein a clipped *P[FRT]* insertion is alternatively moved to another chromosome from said *Drosophila* clipped *FRT* (cFRT) chromosome by treating said *Drosophila* cFRT chromosome with one of mutagens and X-ray.
14. The method according to claim 1, wherein said *Drosophila* cFRT chromosome is alternatively used to establish a *Drosophila* cell line based on a genetic background of said *Drosophila* cFRT chromosome.
15. The method according to claim 1, wherein said *Drosophila* cFRT chromosome is mutated to obtain gene mutations for further experiment.
16. The method according to claim 15, wherein a molecular information of said gene mutations is recovered by retrieving flanking DNA sequences of a clipped *P[FRT]* insertion with a molecular biology method.
17. The method according to claim 16, wherein said molecular biology method includes a plasmid rescue method, a inversed PCR method and a chromosomal walking method.
18. The method according to claim 16, wherein said molecular information of said gene mutations can be recovered by a related bioinformatic manipulation.
19. The method according to claim 18, wherein said related bioinformatic manipulation includes blasting databank, searching gene homologues of biological organisms, analyzing comparative genomics, and analyzing

phylogenic distance and relationship.

20. The method according to claim 15, wherein the functional description of said gene mutations are further analyzed based on the information obtained from said molecular biology method and said related bioinformatic manipulation by using a biological technique.

21. The method according to claim 1, wherein said *Drosophila* cFRT chromosome is used to study the *Drosophila* genes located on the second chromosome and their corresponding gene homologues of other biological organisms including vertebrates, invertebrates, eukaryotes and prokaryotes.

22. A method for generating a *Drosophila* clipped  $FRT^{2L2R}$  (cFRT<sup>2L2R</sup>) chromosome insensitive to a *P* transposase but remaining functional to a yeast site-specific flippase recombinase (FLP), comprising steps of:

- (a) exposing a double-*FRT* chromosome to said *P* transposase for occurring a local and imprecise transposition, wherein said double-*FRT* chromosome contains a first *P*[*FRT*] insertion with a first selection marker gene on one arm thereof and a second *P*[*FRT*] insertion with a second selection marker gene on the other arm thereof;
- (b) screening respectively said first *P*[*FRT*] insertion and said second *P*[*FRT*] insertion insensitive to said *P* transposase to obtain screened products;
- (c) selecting candidate products from said screened products by further examinations; and
- (d) exposing said candidate products by said *P* transposase and selecting a desired product by said further examinations to obtain said *Drosophila* clipped  $FRT^{2L2R}$  (cFRT<sup>2L2R</sup>) chromosome insensitive to

said *P* transposase but remaining functional to yeast site-specific  
flippase recombinase.

23. The method according to claim 22, wherein said method further comprises a  
step (e) of examining the actual molecular nature of said clipped insertions  
by PCR.

24. The method according to claim 22, wherein said step (b) further comprises  
steps of:

(b1) screening said first *P[FRT]* insertion insensitive to said *P*  
transposase subject to an immobility of said first selection marker  
gene; and

(b2) screening said second *P[FRT]* insertion insensitive to said *P*  
transposase from said screened products of step (b1) subject to an  
immobility of said second selection marker gene.

25. The method according to claim 22, wherein said step (b) further comprises  
steps of:

(b1') screening said second *P[FRT]* insertion insensitive to said *P*  
transposase subject to an immobility of said second selection marker  
gene; and

(b2') screening said first *P[FRT]* insertion insensitive to said *P*  
transposase from screened products of step (b1') subject to an  
immobility of said first selection marker gene.

26. The method according to claim 22, wherein said step (c) further comprises  
steps of:

(c1) examining said screened products for both recombination capability  
and homozygous viability; and

(c2) examining recombination accessibility of FRT sequences contained

in said *P[FRT]* insertion by the presence of said FLP to obtain said candidate products.

27. The method according to claim 22, wherein said first selection marker is different from said second selection marker.

28. The method according to claim 22, wherein said *Drosophila* clipped  $FRT^{2L2R}$  chromosome is alternatively generated from two *Drosophila* clipped *FRT* (cFRT) chromosomes ( $cFRT^{2L}$  and  $cFRT^{2R}$  chromosomes) by a genetic recombination method.

29. A *Drosophila* clipped *FRT* (cFRT) chromosome, wherein said chromosome is insensitive to a *P* transposase but remains functional to a yeast site-specific flippase recombinase (FLP), comprising:

a *Drosophila* second chromosome main body; and

a clipped *P[FRT]* (cFRT) insertion immobilized by said *P* transposase.

30. The *Drosophila* cFRT chromosome according to claim 29, wherein said cFRT is formed due to a target sequence, recognized by said *P* transposase and responsible for a *P* transposase transposition, which is damaged and alternated into a type of incomplete target sequence, through one of a group consisting of:

(1) missing of a P5' DNA sequence region;

(2) missing of a P3' DNA sequence region; and

(3) missing of DNA sequences other than those defined in item (1) and in item (2).

31. A *Drosophila* clipped  $FRT^{2L2R}$  ( $cFRT^{2L2R}$ ) chromosome, wherein said chromosome is insensitive to a *P* transposase but remains functional to a yeast site-specific flippase recombinase (FLP), comprising:

- a *Drosophila* second chromosome main body; and
- a clipped *P[FRT]* (cFRT) insertion on a right arm (cFRT<sup>2R</sup>) of said *Drosophila* second chromosome and a clipped *P[FRT]* (cFRT) insertion on a left arm (cFRT<sup>2L</sup>) of said *Drosophila* second chromosome, wherein both said cFRT<sup>2R</sup> and said cFRT<sup>2L</sup> are immobilized by said *P* transposase.
32. The *Drosophila* cFRT<sup>2L2R</sup> chromosome according to claim 31, wherein said *P[FRT]* insertions on a left arm is inserted into the 3' end of the base T at 240696 bp of the AE003781 clone with the P3' end facing the centromere before being clipped, and said *P[FRT]* insertion a right arm is inserted into the 3' end of the base T at 11497 bp of the AE003789 clone with the P5' end pointing toward the telomere before being clipped.
33. The *Drosophila* cFRT<sup>2L2R</sup> chromosome according to claim 31, wherein said cFRT<sup>2L</sup> is an imprecise excision caused by a removal of P5' region and most part of a selection marker gene thereon, wherein a fragment from bases 26 to around 2070 of FBtp0000348 locus is deleted.
34. The *Drosophila* cFRT<sup>2L2R</sup> chromosome according to claim 31, wherein said cFRT<sup>2R</sup> is an imprecise excision caused by a removal of most of the P5' region and one of the FRT DNA repeats, wherein a fragment from bases 10 to 2821 of FBtp0000268 locus is deleted.